# Bromide as a Tracer for Nitrate Uptake in Alfalfa

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## Introduction

New alfalfa germplasms that reduce nitrate ($NO_3$) losses to ground water and decrease fertilizer N requirements in crop rotations would be environmentally and economically beneficial to sustainable cropping systems. Although it has been demonstrated that alfalfa can absorb $NO_3$ from deep in the soil profile, there has been no economically feasible way to select alfalfa plants for differences in $NO_3$ uptake. Selection of alfalfa with high $NO_3$ absorption capacity under greenhouse conditions has failed to produce lines that differ under field conditions. The stable tracer, $^{15}N$, is too expensive to use in the field, where thousands of individual plants must be measured individually.

Bromide (Br) has been used to trace nitrate movement in soil-water systems and is absorbed by many plant species. Our hypothesis was that Br could serve as an inexpensive tracer for $NO_3$-N uptake in $N_2$-fixing crops like alfalfa. We first developed a rapid means of extracting and analyzing Br from alfalfa tissue and then conducted three experiments in the greenhouse to test the hypothesis.

## Methods

We tested several methods of Br extraction from alfalfa tissue, using flow injection analysis and a colorimetric detection technique for quantification. Results were compared to X-ray fluorescence spectroscopy analysis of finely ground tissue samples. We found that a 1 min. extraction in 25 mL deionized water with 0.25 g ground alfalfa tissue and 2 g activated charcoal produced Br analyses that correlated well with XRFS ($r^2 = 0.97$). This rapid extraction procedure removed only part of the total Br, so it is useful only in identifying differences among plants, not in determining total Br uptake.

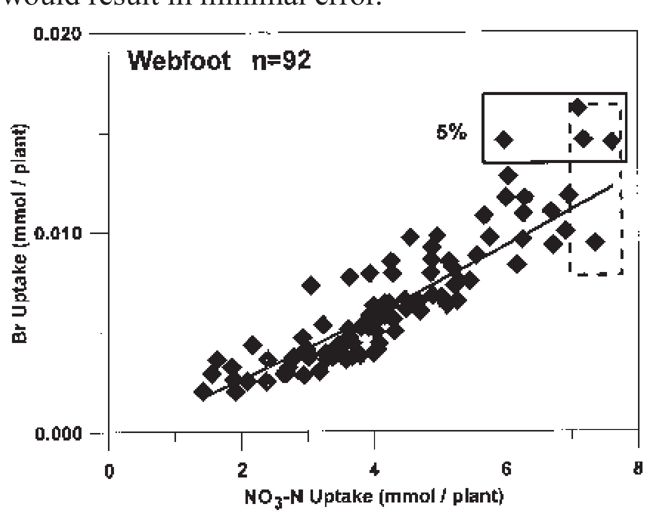
Three experiments were conducted in the greenhouse with established alfalfa plants grown in soil/sand mixtures or sand only, and specific treatments within each experiment were repeated with other plants the following year. Experiment 1 was designed to test the hypothesis that the uptake of Br and N derived from $NO_3$ (using $^{15}N$ to monitor actual uptake of $NO_3$ ) would be related to the concentration supplied, and that the molar ratio of the tracer in the plant would reflect the ratio in solution. Four tracer concentrations at a constant molar ratio of 200 $NO_3$:Br were applied for one regrowth period. Experiment 2 was designed to test the hypothesis that Br uptake would be related to Br concentration in solution applied, regardless of a high concentration of $NO_3$ in solution. We applied four (yr 1) or 6 (yr 2) concentrations of Br with 5 m*M* $NO_3$ for one regrowth period. The third experiment was designed to test the hypothesis that plant-to-plant differences in $NO_3$ uptake are present in alfalfa populations. Solutions containing 5 m*M* $NO_3$ and 0.025 m*M* Br were applied for one regrowth period to 92 plants of Webfoot alfalfa in yr 1 and to 26, 29, and 34 plants of Webfoot, Agate, and Ineffective Agate alfalfa, respectively, in yr 2. Standard analysis of variance or regression analysis techniques were used to evaluate treatment effects.

## Results and Discussion

Both Br and $NO_3$-N uptake increased during regrowth, as expected, due to dry mass increases in the shoot. Nitrate uptake responded in a sigmoidal fashion to increases in $NO_3$ concentration in the root zone, but Br uptake showed a linear response in the range of Br concentrations tested. These differences in uptake pattern and the disparity in physiological role of N and Br in the plant prevent the use of Br to trace $NO_3$-N uptake in crop management or physiology experiments.

The molar ratio of $NO_3$:Br in the shoot tissue closely reflected the changing ratio in nutrient solution, regardless of the amount of time allowed for regrowth (Fig. 1). The one exception to this occurred when 5 micro*M* Br was supplied with 5 m*M* $NO_3$ (yr 2), and these data were not included in the regressions. Because these data apparently fit power functions, the intercept differences in Figure 1 indicate that germplasms responded differently to low ratios of $NO_3$:Br; the higher the apparent intercept in this log-log plot, the higher the initial slope of the response function. When three alfalfa germplasms were tested in yr 2, both of the $N_2$-fixing types, Webfoot and Agate, had similar intercepts, but these were lower than the non-$N_2$-fixing Ineffective Agate, indicating that Ineffective Agate accumulated more $NO_3$ than Br as the $NO_3$:Br ratio increased. Differences in apparent intercept among harvest dates in yr 1 suggest that relatively lower preference is shown for $NO_3$ uptake than Br as alfalfa regrows. In only one harvest (25 d of regrowth in yr 1) was the slope of the relationship different from the others.

Plant-to-plant variability for both $NO_3$-N and Br uptake was evident within each entry in both years. Plants that were high in $NO_3$-N uptake also had high Br uptake (Fig. 2). A hypothetical selection pressure of 5% for the population of 92 plants in yr 1 is shown in Fig. 2. These results show that individual plant selection for $NO_3$-N uptake using Br uptake as the selection criterion would result in minimal error.




**Conclusion**

Results of these experiments suggest that Br has promise as a tracer for $NO_3$-N uptake in an alfalfa plant breeding program. Selection should be made with plants of uniform maturity after several weeks of regrowth to allow differences to be expressed. In addition, a relatively constant supply of Br is necessary because Br uptake is directly related to Br concentration in the root zone. Our technique provides the first affordable means to select alfalfa for $NO_3$-N uptake in the field.

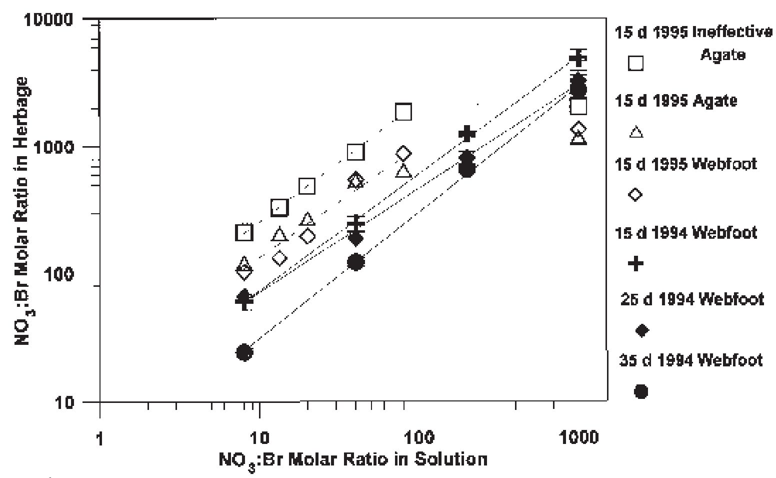



*Figure 1. Molar ratio of $NO_3$-N:Br response to increasing Br concentrations and constant $NO_3$-N concentrations in solution treatments in two years, plotted on a log-log scale. Regression equations had the form $y = ax^b$, with b = 0.94 for all cases except 25 d regrowth in 1994, where b = 0.82 (in all cases $R^2 > 0.85$).*

*Figure 2. Scatter plot of $NO_3$-N uptake vs. Br uptake in yr 1. Selection for 5% of the population using Br uptake compared to $NO_3$-N uptake is shown in the outlined boxes. Regression equation was $y = 1.17(\log x)-6.77$, $r^2 = 0.79$.*